PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/27	A1	(11) International Publication Number: **WO 95/35116** (43) International Publication Date: 28 December 1995 (28.12.95)

(21) International Application Number: PCT/IT94/00086

(22) International Filing Date: 17 June 1994 (17.06.94)

(71) Applicant *(for all designated States except US)*: APPLIED RESEARCH SYSTEMS [NL/NL]; ARS Holding N.V., John B. Gorsiraweg 14, NL-Curacao (AN).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: SAMARITANI, Fabrizio [IT/IT]; Via Luigi Chiala, 130, I-00139 Rome (IT).

(74) Agent: VANNINI, Mario; Istituto Farmacologico Serono SpA, 4th floor, Via Gregoriana, 38, I-00187 Rome (IT).

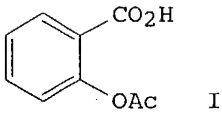
(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: HGH CONTAINING PHARMACEUTICAL COMPOSITIONS

(57) Abstract

Pharmaceutical compositions containing hGH stabilized by means of saccharose. The formulation is particularly suitable for stabilizing a lyophilisate of recombinant hGH.



I

AB Normal subjects and patients with adult-onset diabetes received 10 gm of aspirin (I) [50-78-2] in 4 days. On the fourth day, the fasting serum glucose and the glucose response to oral glucose were decreased in both groups. These changes were assocd. with increased levels of serum insulin [9004-10-8] and pancreatic glucagon [9007-92-5], although the glucagon responses to oral glucose were unchanged. In the diabetic patients, I therapy was followed by a decreased glucose response to i.v. glucose and by the appearance of an early insulin peak, which could not be demonstrated before treatment. I did not affect the i.v. glucose tolerance in normal subjects, although it did enhance the early insulin peak. A decrease in the fasting levels of free fatty acids was noted in both groups, whereas the fasting level of triglycerides decreased only in the diabetic patients. Cholesterolemia did not change in either group. In normal subjects, ibuprofen [15687-27-1] and ketoprofen [22071-15-4], two other presumed prostaglandin inhibitors, did not affect fasting glycemia, glucose tolerance, or the insulin response to glucose.

ST aspirin blood sugar diabetes; insulin **diabetes aspirin** ; glucagon **diabetes aspirin**

IT Diabetes mellitus
(aspirin effect on glucagon and insulin secretion and blood sugar in)

IT Blood sugar
(aspirin effect on, in diabetes, glucagon and insulin secretion in relation to)

IT 50-78-2 15687-27-1 22071-15-4
RL: BIOL (Biological study)
(glucagon and insulin secretion and blood sugar response to, in diabetes)

IT 9004-10-8, biological studies 9007-92-5, biological studies
RL: BIOL (Biological study)
(secretion of, aspirin effect on, in diabetes, blood sugar in relation to)

=>

1999
DOCUMENT TYPE: Abstract PUBLICATION FORMAT: Magazine/Journal; Refereed
ISSN: 0012-1797 LANGUAGE: English RECORD TYPE: Fulltext
TARGET AUDIENCE: Professional
WORD COUNT: 420 LINE COUNT: 00035

4/3/58 (Item 58 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01780625 SUPPLIER NUMBER: 20806525 (USE FORMAT 7 OR 9 FOR FULL TEXT)
The amazing aspirin. (benefits of aspirin)(includes related article on aspirin dosages)
Hudler, Ad
Better Homes and Gardens, v76, n7, p98(4)
July,
1998
PUBLICATION FORMAT: Magazine/Journal ISSN: 0006-0151 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Consumer
WORD COUNT: 1904 LINE COUNT: 00158

4/3/60 (Item 60 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01763509 SUPPLIER NUMBER: 20574458 (USE FORMAT 7 OR 9 FOR FULL TEXT)
ADA recommends aspirin as primary prevention for first MI. (American Diabetes Association; Myocardial Infarction)
Geriatrics, v52, n12, p17(2)
Dec,
1997
PUBLICATION FORMAT: Magazine/Journal; Refereed ISSN: 0016-867X
LANGUAGE: English RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Academic
WORD COUNT: 346 LINE COUNT: 00032

4/3/78 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0012606707 BIOSIS NO.: 200000325020
Preventive aspirin treatment of streptozotocin induced diabetes: Blockage of oxidative status and revertion of heme

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

HGH CONTAINING PHARMACEUTICAL COMPOSITIONS

****

The present invention concerns human growth hormone (hGH) containing pharmaceutical compositions. More precisely, it concerns compositions of saccharose-stabilized human growth hormone. It is known that the highly purified proteins are time-unstable and are stabilized, for instance, in admixture with saccharides, such as lactose and mannitol, or else with proteins and aminoacids, such albumin and glycin.

Human growth hormone is secreted in the human pituitary. In its mature form it consists of 191 amino acids, has a molecular weight of 22,000 and thus is more than three times as large as insulin. This hormone is a linear polypeptide containing two intrachain disulfide bridges. Until the advent of recombinant DNA technology, hGH could be obtained only by laborious extraction from a limited source: the pituitary glands of human cadavers. The consequent scarcity of substance limited its application to treatment of hypopituitary dwarfism even though it has been proposed to be effective in the treatment of burns, wound healing, dystrophy, bone knitting, diffuse gastric bleeding and pseudarthrosis. HGH can be produced in a recombinant host cell, in quantities which would be adequate to treat hypopituitary dwarfism and the other conditions for which it is effective.

The major biological effect of hGH is to promote growth. The organ systems affected include the skeleton, connective tissue, muscles and viscera such as liver, intestine and kidneys. Growth hormone exerts its action through interaction with specific receptors on cell

membranes.

Compositions of lyophilised proteins are described in M.J. Pikal, Biopharm, October 1990, 25-30. There are reported examples of growth hormone formulations with stabilizing excipients such as mannitol, glycin, arginine and lactose.

In particular, the lyophilisation is described in the presence of various substances in their amorphous state, as sugars, which increase the collapse temperature and permit to obtain shorter lyophilisation times. However, it is not feasible, according to the author, to foresee a standard formulation for all the proteins, and the choice of the best formulation requires a remarkable selection work.

German patent DE 3520228 describes bioactive proteins, including growth hormone, in formulations which are stabilized by means of polysaccarides comprising repetitive maltotriose units.

WO 89/09614 describes formulations of human growth hormone stabilized with glycine, mannitol and a buffer, wherein the molar ratio of human growth hormone:glycine is 1:50-200.

US 5122367 patent describes a controlled release system for administration of growth hormones, which comprises the protein and a polysaccaride incorporated within a polymeric matrix.

EP 210039 patent application describes a controlled release implant for subcutaneous administration to an animal of bovine or porcine growth hormone, in the form of a matrix containing 40% saccharose.

According to the present invention, hGH may be either natural or synthetic, i.e. produced on the basis

of recombinant DNA technology, the latter being preferred.

The injectable formulations of human growth hormone are obtained by a process which includes their lyophilisation in order to obtain a dry powder. Human growth hormone is highly liable to denaturization during the lyophilisation process and it desirable to obtain stable formulations to maintain a longer cycle life when they are stored at room temperature.

In order that materials like hGH be provided to health care personnel and patients, these materials must be prepared as pharmaceutical compositions. Such compositions must maintain activity for appropriate periods of time, must be acceptable in their own right to easy and rapid administration to humans, and must be readily manufacturable. In many cases pharmaceutical formulations are provided in frozen or in lyophilized form. In this case, the composition must be thawed or reconstituted prior to use. The frozen or lyophilized form is often used to maintain biochemical integrity and the bioactivity of the medicinal agent contained in the compositions under a wide variety of storage conditions, as it is recognized by those skilled in the art that lyophilized preparations often maintain activity better than their liquid counterparts. Such lyophilized preparations are reconstituted prior to use by the addition of suitable pharmaceutically acceptable diluent(s), such as sterile water for injection or sterile physiological saline solution, and the like.

Alternatively, the composition can be provided in liquid form appropriate for immediate use. Desirable is a liquid formulation which maintains its activity in long term storage.

Current formulation of hGH lose activity due to formation of dimer and higher order aggregates (macro range) during formulation processing as well as during storage and reconstitution. Other chemical changes, such as deamidation and oxidation may also occur upon storage.

Human growth hormone is found on the market in formulations stabilized for example with mannitol Saizen^{R} and Grorm^{R} , Serono.

We have now found that saccharose confers a better stability to the formulation of hGH and in particular to the form of this glycoprotein which has been prepared with the recombinant DNA technique. It has also been found that saccharose unexpectedly prevents the formation of a precipitate when the reconstituted solutions are shaken.

The main object of the present invention is to provide pharmaceutical compositions comprising a solid intimate mixture of human growth hormone and a stabilizing amount of saccharose, alone or in combination of other stabilizing agents.

A further object is to provide a process for the preparation of said pharmaceutical composition, comprising the step of lyophilising an aqueous solution of the components in the containers. Another object is to provide a presentation form of said pharmaceutical composition comprising the said solid mixture hermetically closed in a sterile condition within a container suitable for storage before use and suitable for reconstitution of the mixture for injectable substances.

An other object is to provide a solution for said solid mixture reconstituted into an injectable solution.

In order to evaluate the excipient's effect on the stability of the active ingredients, various formulations of recombinant hGH containing 5 or 10 mg pro vial have been prepared with various excipients: saccharose, glycin, mannitol, saccharose plus mannitol and mannitol plus glycin.

The compositions of the various formulations which have been prepared are reported in tables 1 and 4. The preparation of the lyophilisate was performed by diluting the bulk of hGH with solutions containing the stabilizers all of which in buffers at pH 7,5. The obtained solutions were filtered, brought to the final volume, filled into the various glass vials and lyophilized.

The study of the stability of such formulations stored at 4°C, 25°C, 37°C and 50°C for 24 weeks, was determined through: reverse phase HPLC (RP-HPLC) according to the method described by R.M. Riggin et al., Anal. Biochem., 167:199-209, 1987, and size exclusion HPLC (HPSEC) according to US Pharmacopeia Preview Nov-Dec 1990 pag. 1253-1261. The results are reported in tables 2-3 and 5-6 where the measure is expressed as per cent recovery of hGH in the various formulations.

The chromatographic assay methodology to evaluate the per cent recovery of hGH was carried out as described by Pikal in Pharmaceutical Research 8, pag. 428 "Assays".

In the preformulation phase the effect of pH and of the buffer on the stability of the rhGH on freeze dried form was tested by evaluating the stability at 50°C. Tests were carried out on different buffer systems prepared with acetic acid, phosphoric acid, succinic acid 0.01 M at pH 6.00, 7.00 and 8.00 with NaOH.

The results showed that the rhGH stability was not affected by the buffer, the formulations were anyway more stable at about pH 8.00.

The selected pH for compositions was 7,5.

Seven freeze dried formulations at rhGH concentration of 5 mg/vial were then prepared, using both phosphate and succinate buffer at pH 7,5 to test the compatibility of the active drug with different excipients (saccharose 68.4 mg/vial, mannitol 36,4 mg/vial, mannitol/glycine 25+4 mg/vial, mannitol/saccharose 32+7,5 mg/vial). The amount of excipients was selected in order to have an isotonic solution after reconstitution with bacteriostatic solvent. The filling volume was 1 ml.

Samples, prepared under sterile conditions, were stored at 50°C, 37°C, 25°C and 4°C for 24 weeks and tested by HPSEC, Reverse phase HPLC. PH and moisture content were determined.

The stability of the reconstituted solutions with 0.3% m-cresol and 0.9% benzyl alcohol at 4°C and 25°C was also studied.

The HPSEC and RP HPLC were performed as described before.

The pH was determined by pHmeter on one vial reconstituted with 1 ml of water for injection.
To determine the moisture content of the lyophilized vials, the composition of one vial was suspended in 1 ml of 2-isopropanol, filtered through an Anotop 10, 0,22 um Disposable filter (Merck) and injected in Metrohm Coulometer.

The results of stability, tested by RP-HPLC (Riggin's method), are reported in Table 2. The chromatographic profiles of the formulations containing

saccharose (HGH/3 and HGH/7 of Table 1) after 24 weeks at 50°C are not different from those obtained at time zero.

At the same temperature a purity decrease of 13 - 22% was found in the formulations containing mannitol and mannitol+glycine.

Data reported in table 3 refer to the results obtained by HPSEC analyses. No decrease of rHGH purity percentage was found in all the tested formulations. No significant variation of the moisture content was observed during the study in all lyophilized tested formulations.

A decrease of pH was observed at 37°C and 50°C for lots HGH/5 and HGH/7 of Table 1.

The stability of the reconstituted solutions was also studied tnrough RP-HPLC (Riggin's method) and HPSEC analyses.

With RP-HPLC method, after five weeks at 25°C the purity decrease was found to be in the range of 30% - 50% for samples reconstituted both with benzyl alcohol and with m-cresol. After seven weeks at 4°C the variation was of about 14%in presence of benzyl alcohol and 4% - 8% with m-cresol.

No variation was observed at 4°C with HPSEC method; on the contrary a decrease of rHGH purity of about 5% was found at 25°C for all the formulations in presence both of benzyl alcohol and m-cresol.

Results showed that formulations containing saccharose and saccharose+mannitol presented a better stability profile when compared to the other formulations.

On the basis of the results obtained with the 5mg compositions, saccharose and mannitol were chosen for

the preparation of five freeze dried formulations (Table 4) contained 10 mg hGH/vial using phosphate and succinate buffer at pH 7,5 adjusted with NaOH 2,5 M. One formulation contained 68,4 mg/vial of saccharose (filling volume 1 ml) in phosphate buffer only, the others containing 102,6 mg/vial of saccharose (filling volume 1,5 ml) and mannitol+saccharose 130+40 mg/vial (filling volume 1,5 ml), both in phosphate and succinate buffer. The optimal ratio between saccharose and mannitol and the filling volume to obtain a product with good physical characteristics was adjusted on the basis of preliminary freeze drying trials. The optimum ratio mannitol/saccharose in terms of freeze dried-cake resistance to high temperature was 3:1 and the maximum volume to be freezed dried was 1,5 ml.

The formulations were submitted to stability tests by storing samples at 50°C, 37°C, 25°C and 4°C for 24 weeks. Samples were submitted to the following analytical controls:
HPSEC, RP-HPLC (Riggin's method), pH and moisture content.

The stability of the reconstituted solutions with 0,3% m-cresol and 0,9 % benzyl alcohol at 4°C was monitored for 4 weeks.
Samples were submitted to the same controls performed on the 5mg dosage as described before.
The analyses showed the following results:
The formulations containing 68,4 mg/vial and 102,6 mg/vial of saccharose in succinate buffer tested by RP-HPLC analyses, did not show decrease of purity after 24 weeks storage at all the tested temperatures the results are reported in Table 5.
The formation of degradation products was observed in

the other formulations even after 4/6 weeks storage at 50°C.

No decrease of rHGH purity percentage was found in all tested formulations by HPSEC analyses, see Table 6. During the study no variation of pH and moisture content was observed in all the tested formulations.

Studies on the reconstituted solutions containing only saccharose were also performed by RP-HPLC (Riggin's method) and HPSEC analyses.

After 4 weeks at 4°C, with RP-HPLC method, the purity decrease was found to be of about 12% in presence of benzyl alcohol and 4% with m-cresol.
No decrease of rHGH purity was observed at 4°C, with HPSEC method, in presence both of benzyl alcohol and m-cresol.

To valuate the efficacy of antimicrobial preservation, vials of HGH/3 formulation of Table 1, were reconstituted with 1 ml of bacteriostatic solvent (m-cresol 0,3% or benzyl alcohol 0,9 %). They were tested according to European Pharmacopeia up to 21 days from seeding. Results are reported in tables 7 and 8.

The minimum acceptable efficacy (Minimum criteria) was reached for both the preservative solutions. The results obtained at zero time, in which the microorganisms were counted after spiking in both saline (NaCl 0,9%) and bacteriostatic solution, seem to indicate a higher efficacy of m-cresol vs benzyl alcohol mainly for Staphylococcus and pseudomonas that were reduced immediately after spiking from 90.000 to 25.000 and from 78.000 to 8.000 UFC/ml, respectively (Table 7).

Furthermore, the Aspergillus disappeared in m-cresol after 14 days from seeding (Table 8) and

Pseudomonas after 6 hrs.

The above results indicate that the formulation containing 68,4 mg of saccharose, phosphate buffer at pH 7,5, filling volume 1 ml reconstituted with meta-cresol 0,3% is the one that guarantees the best stability of r-HGH both at 5 and 10 mg strength.

EXAMPLE OF PHARMACEUTICAL MANUFACTURING

Materials: pure saccharose Ph Eur, BP, Nord, NF (mercK); H_3PO_4 Suprapur (Merck); NaOH for analysis use (Merck); water for injectable.

As containers have been used vials DIN 2R (borosilicate glass type I) , rubber closures (Pharmagummi W1816 V50) and Alluminium rings and Flip-off caps (Pharma-Metal GmbH).

Preparation of rHGH solution containing saccharose (for 1000 vials containing each 10 mg hGH).

Saccharose (68,4 g), H_3PO_4 (1,96 g) are dissolved into water for injectables (800 ml) in order to obtain the starting saccharose solution.

The bulk of the hGH (10 g) is added to the saccharose solution that, after the pH has been adjusted at 7,5 by means of 2,5 M NaOH, is brought to the final volume of 1000 ml. The solution is filtered through a 0,22 um Durapore sterile filter (Millipore). During the process the solution temperature is kept between 4° and 8°C. The solutions containing different excipients or a different active drug dosage have been prepared in a similar manner.

Filling up and lyophilisation

The vials are filled up with 1 ml of HGH sterile solution , transferred to the freeze dryer and cooled at -45°C for 6 hrs. at least. The lyophilisation is started

at the temperature of -45°C with a vacuum of 0,07 mBar. The heating is performed according to the following scheme:+10°C for 12 hrs.; then +35°C until the end of the cycle.

5

Table 1

5 mg VIAL COMPOSITION

	HGH/1	HGH/2	HGH/3	HGH/4	HGH/5	HGH/6	HGH/7
Components:							
r-HGH mg/vial Lot. n. PGRR9201D1	5	5	5	5	5	5	5
Saccharose mg/vial	7.5	–	68.4	–	7.5	–	68.4
Mannitol mg/vial	32	36.4	–	25	32	36.4	–
Glycine mg/vial	–	–	–	5	–	–	–
Buffer:							
Phosphoric Acid mg/vial	0.98	0.98	0.98	0.98	–	–	–
Succinic Acid mg/vial	–	–	–	–	1.18	1.18	1.18
NaOH q.s. to pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Filling volume	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Reconstitution volume	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

Table 2

SAIZEN 5 mg

r-HGH CHROMATOGRAPHIC PURITY by RIGGIN'S METHOD FREEZE-DRIED FORMULATIONS

		4°C					25°C			37°C				50°C				
	T=0	1 W	2 W	4 W	8 W	24W	4 W	8 W	24W	1 W	4 W	8 W	24W	1 W	2 W	4 W	8 W	24W
HGH/1 (M/S)	94.63	95.65	---	---	94.51	95.74	94.35	94.71	95.83	95.07	95.15	93.53	---	94.95	94.45	93.62	92.6	92.86
HGH/2 (M)	94.75	96.09	---	---	90.59	92.13	94.32	92.97	92.21	94.09	92.48	88.83	---	92.05	89.00	87.97	83.41	72.3
HGH/3 (S)	94.44	95.38	---	---	95.16	96.28	94.66	94.98	96.41	95.10	95.52	94.43	---	95.06	95.04	94.4	94.66	95.69
HGH/4 (M/G)	94.57	95.40	---	---	93.14	93.65	94.56	93.59	93.98	94.59	93.85	90.81	---	93.09	91.72	88.78	85.89	72.22
HGH/5 (M/S)	94.25	95.45	---	---	94.04	95.02	94.77	94.28	94.59	94.56	94.39	92.57	---	94.10	93.09	92.34	91.93	90.27
HGH/6 (M)	94.29	95.50	---	---	92.50	93.74	94.09	93.56	94.13	93.99	91.74	91.11	---	92.65	91.25	89.58	87.94	81.62
HGH/7 (S)	94.15	95.00	---	---	95.37	96.15	---	94.99	96.46	94.55	94.39	94.52	---	94.67	94.93	94.49	94.54	95.70

SUBSTITUTE SHEET

M/S = Mannitol +Saccharose

M = Mannitol

M/G = Mannitol + Glycine

S = Saccharose

W = Week

Table 3 rHGH CHROMATOGRAPHIC PURITY by HPSEC

FREEZE-DRIED FORMULATIONS

		4°C					25°C			37°C					50°C					
	T=0	1 W	2 W	4 W	8 W	24W	4 W	8 W	24W	1 W	4 W	8 W	12W	24W	1 W	2 W	4 W	8 W	12W	24W
HGH/1 (M/S)	97.56	97.98	---	---	98.09	98.68	97.24	98.54	98.38	96.94	98.31	98.20	98.32	---	98.24	97.93	98.24	97.76	97.66	98.01
HGH/2 (M)	97.64	97.96	---	---	97.04	98.24	98.11	98.45	98.28	97.30	97.77	98.04	97.07	---	98.05	97.64	97.91	95.19	97.14	97.02
HGH/3 (S)	97.75	97.96	---	---	98.20	98.40	97.15	98.49	98.43	97.70	98.12	98.24	98.52	---	98.29	97.90	98.44	98.06	98.33	98.50
HGH/4 (M/G)	96.55	96.90	---	---	96.10	98.29	98.14	98.48	98.60	96.58	98.16	97.96	97.26	---	98.40	98.12	98.26	96.52	96.46	96.82
HGH/5 (M/S)	97.41	97.99	---	---	98.28	98.35	97.88	98.50	98.61	97.67	98.25	98.15	98.08	---	98.24	98.09	98.44	98.12	98.00	98.20
HGH/6 (M)	97.45	97.99	---	---	97.84	97.86	98.07	98.52	98.44	97.59	97.86	98.08	98.02	---	98.00	96.85	97.81	96.34	96.98	96.79
HGH/7 (S)	97.60	97.94	---	---	98.31	98.47	98.10	98.47	98.51	97.80	98.09	98.31	98.21	---	98.22	98.26	98.47	98.21	98.11	98.35

M/S = Mannitol +Saccharose

M = Mannitol

M/G = Mannitol + Glycine

S = Saccharose

W = Week

SUBSTITUTE SHEET

Table 4

10 mg VIAL COMPOSITION

	S10/S/F/1/01	S10/S/F/01	S10/S/S/01	S10/SM/F/01	S10/SM/S/01
Components:					
r-HGH mg/vial Lot. n. PGRR9201D2	10	10	10	10	10
Saccharose mg/vial	68.4	102.6	102.6	40	40
Mannitol mg/vial	–	–	–	130	130
Buffer:					
Phosphoric Acid mg/vial	1.98	1.98	–	1.98	–
Succinic Acid mg/vial	–	–	2.36	–	2.36
NaOH q.s. to pH	7.5	7.5	7.5	7.5	7.5
Filling volume	**1 ml**	**1.5 ml**	**1.5 ml**	**1.5 ml**	**1.5 ml**
Reconstitution volume	**2 ml**	**2 ml**	**2 ml**	**2 ml**	**2 ml**

TABLE 5

rHGH CHROMATOGRAPHIC PURITY by RP–HPLC (RIGGIN'S METHOD)

FREEZE–DRIED FORMULATIONS

		4°C			25°C				37°C				50°C			
	T–0	4W	6W	8W	4W	6W	8W	24W	4W	6W	8W	24W	4W	6W	8W	24W
S10/S/S/01	95.87	---	---	96.08	95.61	95.68	95.28	96.60	95.16	95.66	95.93	96.11	94.73	94.99	94.89	93.74
S10/S/F/1/01	95.65	---	---	96.01	95.36	95.65	95.92	94.69	94.91	95.48	95.40	94.62	94.17	94.43	94.45	94.31
S10/S/F/01	95.34	---	---	95.93	95.13	95.57	95.08	---	94.32	94.50	93.41	92.46	92.13	91.47	89.75	---
S10/SM/S/01	95.52	---	---	95.86	95.83	95.32	95.35	93.38	94.20	94.69	94.50	92.83	---	84.81	---	---
S10/SM/F/01	95.23	---	---	95.70	94.44	94.94	95.24	90.04	92.51	92.76	90.74	---	87.92	85.29	82.03	---

S10/S/S/01 = SACCHAROSE/SUCCINATE (FILLING VOLUME 1.5 ml)
S10/S/F/1/01 = SACCHAROSE/PHOSPHATE (FILLING VOLUME 1 ml)
S10/S/F/01 = SACCHAROSE/PHOSPHATE (FILLING VOLUME 1.5 ml)
S10/SM/S/01 = SACCHAROSE+MANNITOL/SUCCINATE (FILLING VOLUME 1.5 ml)
S10/SM/F/01 = SACCHAROSE+MANNITOL/PHOSPHATE (FILLING VOLUME 1.5 ml)

Table 6

rHGH CHROMATOGRAPHIC PURITY by HPSEC

FREEZE-DRIED FORMULATIONS

		4°C			25°C				37°C				50°C			
	T=0	4W	6W	8W	4W	6W	8W	24W	4W	6W	8W	24W	4W	6W	8W	24W
S10/S/S/01	98.20	---	---	97.93	98.41	98.17	98.98	98.27	97.97	98.17	98.09	98.17	97.98	97.84	97.93	98.19
S10/S/F/1/01	98.23	---	---	98.04	98.28	98.34	98.26	98.33	98.28	98.56	98.01	98.24	97.98	98.20	97.87	98.07
S10/S/F/01	98.13	---	---	97.81	98.24	98.17	98.07	---	98.06	98.50	97.90	98.01	97.33	97.82	97.50	98.01
S10/SM/S/01	97.97	---	---	97.82	98.19	98.32	98.22	98.30	96.62	98.14	98.17	97.76	97.61	97.22	97.93	---
S10/SM/F/01	98.17	---	---	97.75	97.98	98.35	98.21	97.61	97.94	98.19	97.26	---	97.34	98.57	98.72	---

S10/S/S/01 = Saccharose/Succinate (filling volume 1.5 ml)
S10/S/F/1/01 = Saccharose/Phosphate (filling volume 1.0 ml)
S10/S/F/01 = Saccharose/Phosphate (filling volume 1.5 ml)
S10/SM/S/01 = Saccharose+Mannitol/Succinate (filling volume 1.5 ml)
S10/SM/F/01 = Saccharose+Mannitol/Phosphate (filling volume 1.5 ml)

Table 7

Efficacy of antimicrobial preservation. Benzyl Alcohol 0.9% was used as antimicrobial preservative (PRES) in hGH formulated vials (SAIZEN).The test was carried out according to the European Pharmacopeia and followed up to 21 days from seeding.The log. reduction was calculated vs the UFC counted at Zero time (ZT) in the preservative solution.

MICRORGANISMS	ZT		6 hrs		24 hrs		7 DAYS		14 DAYS		21 DAYS	
	SALINE UFC/ml	PRES. UFC/ml	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES
STAPHYLOCOCCUS AUREUS	90000	85000	0	>3	80	>3	0	>3	0	>3	0	>3
PSEUDOMONAS AERUGINOSA	78000	48000	18000	0.4	0	>3	0	>3	0	>3	0	>3
CANDIDA ALBICANS	92000	36000	N.T.	—	N.T.	—	0	>3	0	>3	0	>3
ASPERGILLUS NIGER	98000	75000	N.T.	—	N.T.	—	4000	1.3	300	2.4	200	2.6

N.T.= not tested

SUBSTITUTE SHEET

SUBSTITUTE SHEET

Table 8

Efficacy of antimicrobial preservation. M-Cresolo 0.3% was used as antimicrobial preservative (PRES) in hGH formulated vials (SAIZEN).The test was carried out according to the European Pharmacopeia and followed up to 21 daysfrom seeding.The log. reduction was calculated vs the UFC counted at Zero time (ZT) in the preservative solution.

MICRORGANISMS	ZT		6 hrs		24 hrs		7 DAYS		14 DAYS		21 DAYS	
	SALINE UFC/ml	PRES. UFC/ml	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES	UFC/ml	lg RED. vs PRES
STAPHYLOCOCCUS AUREUS	90000	25000	1000	1.4	0	>3	0	>3	0	>3	0	>3
PSEUDOMONAS AERUGINOSA	78000	8000	0	>3	0	>3	0	>3	0	>3	0	>3
CANDIDA ALBICANS	92000	60000	N.T.	—	N.T.	—	0	>3	0	>3	0	>3
ASPERGILLUS NIGER	98000	78000	N.T.	—	N.T.	—	3000	1.4	0	>3	0	>3

N.T.= not tested

CLAIMS

1. A pharmaceutical composition comprising a solid intimate mixture of human growth hormone (hGH) and a stabilizing amount of saccharose, alone or in combination with other excipients.

2. A pharmaceutical composition according to Claim 1, wherein the solid intimate mixture is a lyophilisate.

3. A pharmaceutical composition according to Claim 1, wherein the hGH is recombinant.

4. A pharmaceutical composition according to any of Claims 1 to 3 wherein the stabilizing agent is saccharose alone.

5. A pharmaceutical composition according to any of Claims 1 to 4 wherein the stabilizing agent is saccharose in combination with mannitol.

6. A pharmaceutical composition according to any of Claims 1 to 5, containing 5 or 10 mg/vial of hGH.

7. A pharmaceutical composition according to any of Claims 1 to 6 containing a buffer solution selected from acetate buffer, succinate buffer and phosphate buffer.

8. A pharmaceutical composition according to any of Claims 1 to 7 wherein the buffer solution is phosphate buffer.

9. A pharmaceutical composition according to any of

Claims 1 to 8 wherein the pH of the solution is within the range 6.0 to 8.0.

10. A pharmaceutical composition according to any of Claims 1 to 9 wherein the pH of the solution is 7,5.

11. A pharmaceutical composition according to any of Claims 1 to 10 comprising 5 or 10 mg/vial of hGH, 68,4 mg/vial of saccharose and phosphate buffer at pH 7,5.

12. A process for preparing a pharmaceutical composition according to any of Claims 1 to 11 comprising the preparation of an aqueous solution of the components, the distribution within containers and the lyophilisation in the containers.

13. Forms of presentation of said pharmaceutical composition comprising the solid mixture according to any of Claims 1 to 11, hermetically closed in a sterile condition within a container suited for storage before use and for reconstitution of the mixture into a solvent or into a solution for injectables.

14. A solution comprising the solid mixture according to Claim 13, reconstituted in a solvent or a solution for injectables.

15. A solution according to Claim 14, wherein the solvent is a bacteriostatic solvent.

16. A solution according to Claim 15, wherein the bacteriostatic solvent is m-cresol 0,3%.

INTERNATIONAL SEARCH REPORT

Intc.. ional Application No
PCT/IT 94/00086

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/27

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 12812 (NOVO NORDISK A/S) 8 July 1993 see page 8, line 22 - page 9, line 15 see page 14, line 24 - page 16, line 10	1-16
Y	US,A,5 122 367 (EYAL RON ET AL.) 16 June 1992 cited in the application see column 2, line 49 - column 3, line 26	1-16
Y	WO,A,94 03198 (GENENTECH, INC.) 17 February 1994 see page 4, line 26 - page 7, line 30	1-16
Y	EP,A,0 303 746 (INTERNATIONAL MINERALS AND CHEMICAL CORPORATION) 22 February 1989 see page 3, line 9 - page 4, line 12 see page 4, line 35 - line 56	1-16

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 1995	27.02.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rempp, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte. ional Application No
PCT/IT 94/00086

C.(Contunuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 19773 (PITMAN-MOORE, INC.) 14 October 1993	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter...tional Application No
PCT/IT 94/00086

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9312812	08-07-93	AU-B-	3344693	28-07-93
		CA-A-	2125855	08-07-93
		EP-A-	0618807	12-10-94
		FI-A-	942906	17-06-94
		NO-A-	942300	19-08-94
US-A-5122367	16-06-92	NONE		
WO-A-9403198	17-02-94	AU-B-	4792793	03-03-94
EP-A-0303746	22-02-89	HU-A-	44706	28-04-88
WO-A-9319773	14-10-93	AU-B-	4278393	08-11-93
		CA-A-	2133737	14-10-93
		CN-A-	1078910	01-12-93